Faculty of Resource Science and Technology

# PRENATAL GROWTH AND BREEDING STATUS OF *Cynopterus brachyotis*


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## DECLARATION

I hereby declare that no portion of the work referred to this thesis has been submitted in support of an application for another degree or qualification to this or any other university or institute of higher learning.

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(Rahmat bin Libar)

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| °C | Degree Celcius (temperature) |
| CRL | Crown rump length |
| FAL | Forearm length |
| GLS | Greatest length of skull |
| Km | Kilometre |
| Mm | Millimetre |
| NP | National Park |

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**Prenatal Growth and Breeding Status of *Cynopterus brachyotis***

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## ABSTRACT

*Cynopterus brachyotis* is one of seven species under genus *Cynopterus* (Megachiroptera, Pteropodidae). I studied the prenatal growth and development of this species. I measured the forearm length (FAL), crown rump length (CRL) and greatest length of skull (GLS). Linear regression for general growth trajectories were generated by plotting FAL and CRL against GLS. Both FAL and CRL showed positive relationship with GLS. The embryos of *C. brachyotis* have been successfully staged by referring to recent bat staging system and the expected mating and birthing month were estimated. I estimate the following regarding *C.brachyotis* reproduction. In general, the reproduction of *C.brachyotis* can be characterized as aseasonally polyoestrus. The peak birth season in *C.brachyotis* does not correspond to the season of greater rainfall (Nov-Jan) and fruiting season (March-June). This provides indication on how this species is abundance and successful.

Key words: *Cynopterus brachyotis,* forearm length, crown rump length, greatest length of skull, aseasonally polyoestrus

## ABSTRAK

*Cynopterus brachyotis adalah salah satu daripada tujuh spesies di bawah genus Cynopterus (Megachiroptera, Pteropodidae). Saya mengkaji pertumbuhan pralahir spesis ini. Saya telah mengukur panjang lengan (FAL), panjang bahagian kepala sehingga ke punggung (CRL) dan panjang besar tengkorak (GLS). Regresi sejajar untuk trajektori pertumbuhan umum telah dijana dengan memplot FAL dan CRL terhadap GLS. Kedua-dua FAL dan CRL menunjukkan hubungan positif dengan GLS. Embrio C. brachyotis telah berjaya disusun mengikut peringkat perkembangan dengan merujuk kepada sistem susunan perkembangan embrio yang terbaru. Bulan untuk mengawan dan kelahiran bagi spesies ini juga telah dianggarkan. Saya menganggarkan berikut mengenai pembiakan C.brachyotis. Secara umum, pembiakan C.brachyotis boleh disifatkan sebagai mempunyai kitaran estrus lebih daripada sekali yang tidak bermusim (polyoestrus aseasonally). Puncak musim kelahiran pada C.brachyotis tidak sepadan dengan musim hujan yang lebih tinggi (Nov-Jan) dan musim berbuah (Mac-Jun). Ini memberi petunjuk kepada bagaimana spesies ini boleh menyesuaikan diri dalam apa jua keadaan persekitaran seterusnya menjadikan bilangan mereka bertambah.*

*Kata kunci: Cynopterus brachyotis, panjang lengan, panjamg nahagian kepala ke punggung, panjang besar tengkorak, kitaran estrus lebih daripada sekali yang tidak bermusim.*

## 1.0 Introduction

Mammals occupy most area in the world from terrestrial to aquatic with different stages in development and breeding pattern in which their abundance and survival depend on many factors. The knowledge on development especially for mammals comes from order Rodentia (Theiler, 1972; Dyban *et al.,* 1975; Donkelaar *et al.,* 1979). The knowledge on prenatal development of order Chiroptera is limited, although this order is speciose. This order consists of 18 families and 186 genera of bats around the world (Nowak, 1999). According to Payne *et al.* (1985), there are at least eight bat families present in Borneo Island including Pteropodidae. One of the most abundance species in the family Pteropodidae is *Cynopterus brachyotis*. This is a common species and plays an important ecological role as fruit disperser over a large area (Tan *et al.,* 2000). However, their growth development is not well documented.

The high number of species in ecosystem can be also known by looking on their reproductive pattern. The prenatal growth of *C. brachyotis* has not been previously documented and limited to morphological examination of mother's bats. In general, prenatal growth is the developmental course of the organism starting from fertilization until birth. During intrauterine development, variable organs grow at different rates leading to continuous changes in organisms (Hafez, 1963) and the documentation of the developmental series in *C. brachyotis* embryo is such extra information in embryology study for this species. Jerret (1979) stated that diverse mammals such as Chiroptera may show variable specialization in female reproductive cyclicity and can be categorized in some patterns which are seasonally polyoestrous, aseasonally polyoestrous and seasonally monoestrous. Monoestrous pattern is the organism having one estrous cycle per year, while polyoestrous pattern showing more than one cycles throughout the year. However, the regulation of breeding season might be different in bats which can contribute to their richness in ecosystem despite their survival during postnatal growth. The fruit bats depend much on fruits as their diet and the consumption of enough food during pregnancy is important. It is related to the fetal growth which depending on the nutrition of food as maternal malnutrition can impair the growth of the fetus (Wu *et al.,* 2004). Although *C. brachyotis* is a successful species in Malaysia and well distributed around Southeast Asia, their breeding seasons and embryonic development are poorly studied. Therefore, study on the prenatal growth and breeding status of this species is important to improve our knowledge on embryology and perhaps to give answer to the reproductive success of this species in Southeast Asia.

As no study record on developmental series of embryo for this species, I would like to understand the growth relationship between Crown Rump Length (CRL), Forearm Length (FAL) and body mass relate to the growth of the Greatest Length of Skull (GLS) of *C. brachyotis* embryo. The growth development of embryo can also be seen on the growth of embryo throughout the gestation period and one of known staging system for Chiroptera was reported by Cretekos *et al.* (2005). Therefore, I also would like to know whether the current staging system can fit to *C. brachyotis* embryo development. Other than that, another question that I interest in is the correlation of breeding pattern of *C. brachyotis* in Sarawak with ecological factors.

## 2.0 Literature Review

### 2.1 Bats taxonomy, distribution and diet

Bats can be divided into two suborders which are Megachiroptera (frugivorous and nectarivorous) and Microchiroptera (frugivorous, sangunivorous, omnivorous, carnivorous, nectarivorous, and insectivorous) (Corbet and Hill, 1992; Findley, 1993). Megachiroptera consist only one family which is Pteropodidae, having 42 genera and 166 species over the world (Corbet and Hill, 1992; Nowak 1999). Pteropodidae is further divided into four modern subfamilies, namely Pteropodinae, Harpyionycterinae, Nyctimeninae and Macroglossinae (Corbet and Hill, 1992). Megachiroptera is well distributed around the world throughout old world tropics, ranging from Africa region through Asia continent, Australia and on the island of Indian plus western Pacific Oceans (Mickleburgh *et al.,* 1992). True flight ability in bats allow them to fill many feeding niches and adapted to feed on various food sources such as fruits, leaves, flowers, nectar, pollen, insects, fish, small vertebrates and blood. Recent update by Simmons (2005) showed that there are 1116 recognized species of bats throughout the world and for about 50 percent of them use plants to roost (Kunz and Lumsden, 2003) and some bats roosts are modified by the bats itself and not biotic or abiotic agent (Kunz *et al.,* 1994; Tan *et al.,* 1997). The genus *Cynopterus* F. Cuvier 1824 is also commonly known as Short-nosed Fruit Bats or Dog-faced Fruit Bats which widely distributed in the Indo-Malayan region comprise of four species (Corbet and Hill, 1992). Recently, Simmons (2005) listed 7 species in this genus which are *C. sphinx* (Vahl, 1797); *C. tithaecheilus* (Temminck, 1825); *C. brachyotis* (Müller, 1838); *C. horsfieldii* (Gray,1843); *C. luzoniensis* Peters, (1861); *C. minuta* (Miller, 1906); and *C. nusatenggara* (Kitchener and Maharadatunkamsi, 1991). Abdullah and Jayaraj (2006) reported that there are two forms of *C. brachyotis* which are large and small form and possibility the small form represent a new species. *C. brachyotis* is a common plant-visiting bat in Southeast Asia. Throughout its range, it may occupy variety of habitats including primary or disturbed forest, orchard, mangrove and cultivated area (Corbet and Hill, 1992). This species also can be found in Nepal, southern and probably northeastern India and Guangdong Province of southeastern China (Nowak, 1999). *C. brachyotis* habitually roost in small groups on trees such as under the fronds of palms (Rahman *et al.,* 2011). Mohd-Azlan *et al.* (2010) reported that *C. brachyotis* returned to feed on the same roost on subsequent nights indicating that roosts were not being abandoned. Tan *et al.* (1997) on *C. brachyotis* Sunda and Campbell *et al.* (2006) studied on *C. brachyotis* forest lineage suggest that both species occupy and modify palmate leaves of fan palms. Campbell *et al*. (2006) documented that the use of modified roost is intermittently in *C. brachyotis* Forest and not at all in *C. brachyotis* Sunda. The ability to adapt into many foraging areas and able to feed on many types of fruit in this species are largely influenced by the seasonal flowering and fruiting phenologies of trees (Lim, 1966) where the Peninsular Malaysia and Borneo Malaysia have different terms of floristic, endemism and flowering phonologies. The study on diet by using remote flash photography showed two peak feeding activities in *C. brachyotis* which are before midnight around 1900-2000 hours and between 2200-2300 hours and the bats feed on at least 24 species (18 species of seeds and 6 species of fruits) (Mohd-Azlan *et al.,* 2010). Tan *et al.* (2000) mentioned that *C. brachyotis* consumed 38 plant species and *Calophyllum inophyllum* and *Ficus fistulosa* species are available throughout the years.

## 2.2 Bats reproductive ecology

The ecology of bats reproductive has been reviewed in recent years (Jerrett, 1979; August, and Baker, 1982; Heideman 1988; Rasweiler and Badwaik 1997; Sharifi *et al,*. 2004; Wyant and Adams, 2007; Kofron, 2007a, 2007b; 2008). Some examples of bats which are polyestrous with no breeding season are *Desmodus rotundus* (Desmodontidae) (Wimsatt and Trapido., 1952), and *Taphozous longimanus* (Emballonuridae) (Gopalakrishna, 1955). These bat species will breed at any time suitable without depending on the environment factor at most. Some species showing seasonally polyestrous are *C. brachyotis* (Lim, 1970), *Epomophorus anurus* (Okia, 1974). Study on *Balionycteris maculata* (Kofron, 2007a) and *Penthethor lucasi* (Kofron, 2007b) mentioned that the estimated birthing season for these species occurred during a season of greater rainfall and not timed with flowering/fruiting season and the reproduction pattern showed is seasonally monoestry. While, bimodal polyoestry pattern was shown by *Macroglossus minimus* with the birthing time correlate with two seasons of rainfall (Kofron, 2008). Additionally, body mass is another important aspect in determining the growth of embryo and can also be used in determining the breeding pattern of bats (Okia, 1974). The pregnant female will usually give one infant for every pregnancy (Jerret, 1979; Nowak, 1999) and two fetuses were found in rare instances (Nowak, 1999). The breeding pattern of bats may be in response to the environment as Heideman (1987) stated that the *Haplonycteris* reproduction timing patterns is depend on the response of local seasonal patterns of resource abundance. The pregnancy should be occurred in animals where there are optimum period where abundance resources are available. In Malaysia, the period between March and June are the fruiting season and abundance of fruits from various species which *C. brachyotis* feed such as rambutan (*Naphelium lappaceum*), ciku (*Archras zapota*), guava (*Psidium guajava*), jackfruit (*Artocarpus heterophyllus*) and cempedak (*Arocarpus integer*)

(Lim, 1970). The abundance of food resources also lead to high nutrition intake by the bats mother. Limitation on breeding activities is related to the food sources abundance in order to increase fitness (August and Baker 1982). Nutrition is important as it might affect the intrauterine environment of the bats mother and can give lifelong consequences (Wu *et al.,* 2004). Specifically, in *C. brachyotis*, Lim (1970) showed that there are three pregnancy peaks in a year which are in January, May and September whereas seasonally breeding pattern with two births periods per year in Philippine island was stated by Ingle (1992). The timing of reproductive might be different depending on localities as in Ulu Gombak, pregnancy and lactation vary from year to year and *C. brachyotis* is assumed to have shorter reproductive cycles to produce two to three young each year (Funakoshi and Zubaid, 1997). The breeding cycle of female is depend on the gestation period and it is approximated that this species having 4 months gestation period and the lactation lasts for 6-8 weeks (Heideman, 1987; Nowak, 1999).

### 2.3 Embryo development / Established embryo staging

The gestation period in animals is different varying from 16 days in the golden hamster up to 15-17 months in the rhinoceros or cachalot (Sterba, 1995). The description of prenatal stages might be different in some authors. Most studies on mammals embryology rely on a single group which is order Rodentia (Cretekos *et al.,* 2005), thus creating a gap in knowledge. For examples are Theiler (1972) established 27 stages in the prenatal development of the laboratory mouse where Dyban *et al.* (1975), describe 24 stages in the prenatal development of the laboratory mouse, laboratory rat, the Chinese hamster, *Cricetulus griseus,* and the domestic rabbit, *Oryctolagus cuniculus.* The stages documented are classified by the degree of morphological difference and certain variation range and individual variation (Sterba, 1995) and result in different stages reported by different authors. For example is the experiment by Donkelaar *et al*. (1979) provide 22 stages in the prenatal development of Chinese hamster, *Cricetulus griseus* which is less 2 stages compared to Dyban *et al.* (1975). Detailed studies in Rodentia creating wide information in this order, but by taking the rodents as mammalian model for reproduction and development may be misleading (Cretekos *et al.,* 2005). For chiropteran studies, the developmental series of some important measurements of *Mops condylurus* embryo was reported by Wyant and Adams (2007). Besides, the embryo development of *Carollia perspicillata* based on specimens collected at timed intervals after captive mating was arranged into 24 stages with (Cretekos *et al.,* 2005) and was proposed as bats staging system has been used as a framework for staging bats embryo in pteropodid (*Rousettus amplexicaudatus*; Giannini *et al.,* 2006) and a vespertilionid bat (*Pipistrellus abramus*; Tokita, 2006). This system also has been used in comparison study of three different bat species by Wang *et al.* (2010).

## 3.0 Materials and Method

### 3.1 Study Area

This study was conducted by using most of voucher specimen from UNIMAS zoological museum and some additional collection from wild. *Cynopterus brachyotis* specimens from the museum are from various divisions all over Sarawak as in (Figure 1) and were captured from several years back. Figure 1 below show divisions or location where the bats were captured. Two sampling were done for this project which was in Kubah NP and Bako NP to collect additional specimen. Sarawak is located in Borneo Island and the largest state in Malaysia covering approximately 124,449.5 km$^2$ (16.67 %) out of 746,337 km$^2$ total area of Borneo (MacKinnon *et al.*, 1996).

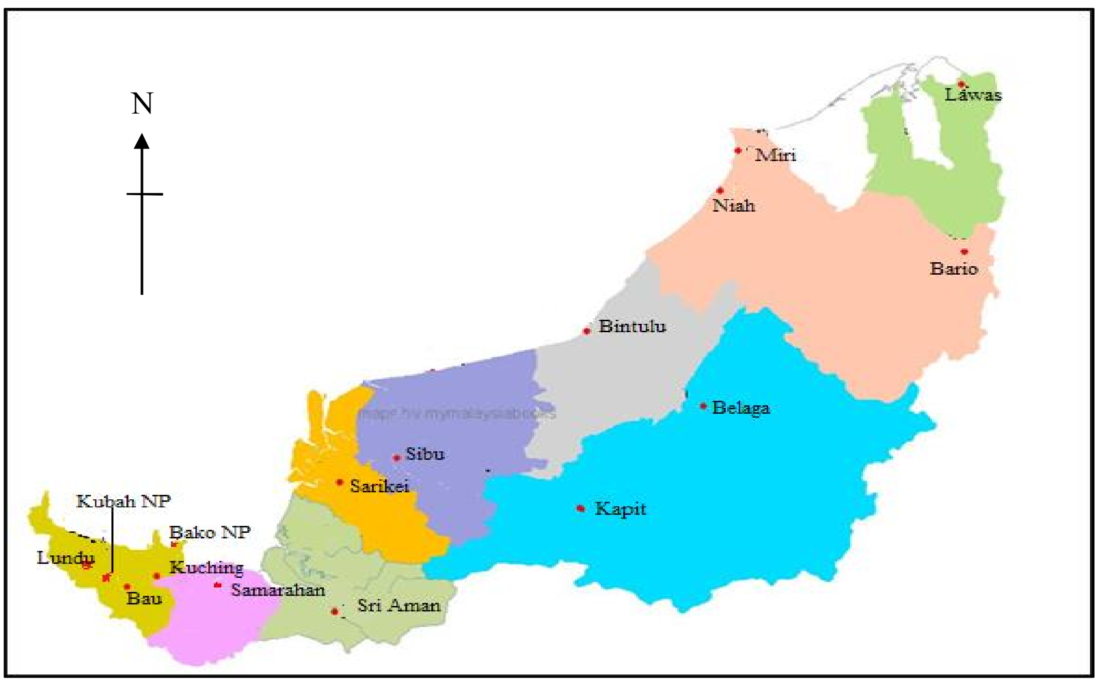


Fig 1. Map of study area. Not according to scale

The data on rainfall and temperature of Sarawak has been pooled from record of five locations which are Kuching, Sri Aman, Kapit, Sibu and Bintulu. The climate of Sarawak can be characterized by inconsistent temperature throughout year, high humidity and rainfall with V-shaped pattern. Generally, Sarawak is having low temperature in January and December with the lowest record is at 30.6 °C (Fig 2). Rainfall in Sarawak is lower in the middle of year, and the highest is in January followed by December and November**.**

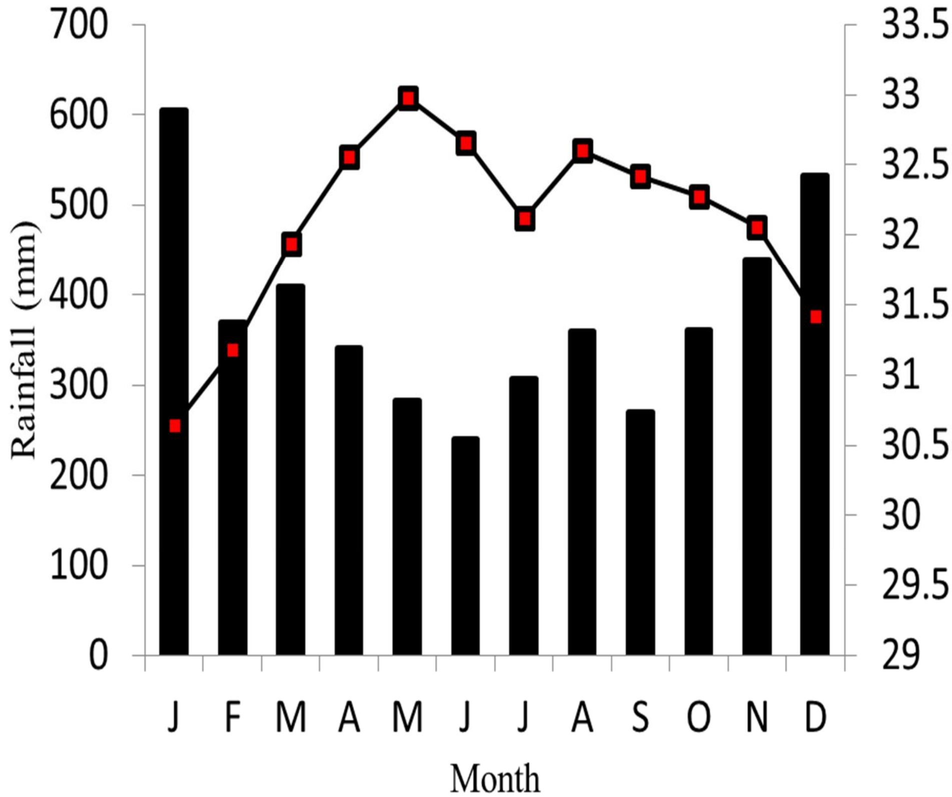


Fig 2. Rainfall and temperature trends in Sarawak. data gathered in year 2008, 2009 and 2010

### 3.1.1 Bako National Park

The trapping was conducted at Bako National Park which located at Muara Tebas Peninsula on the north-eastern part of Kuching, and about 37 kilometers away from Kuching city (Khan *et al.,* 2007). This park consists of seven different major habitats: heath forest, mangrove forest, mixed dipterocarp forest, riverine forest, beach forest, grassland and cliff vegetation, which provides diverse ecological niches for high species richness and diversity of fauna (Hazebroek and Abang Kashim, 2000). The main attractions to this park are some wild animals such as proboscis monkey (*Nasalis larvatus*), the endangered flying lemur (*Galeopterus variegatus*), western tarsier (*Tarsius bancanus*), silvered langur (*Presbytis crystata*) and bearded pig (*Sus barbatus*).

### 3.1.2 Kubah National Park

Kubah NP is located about 22 kilometers from Kuching covering an area approximately 2230 ha and some regions of this park is surrounded by villages and agricultural setting such as banana (*Musa* sp.), durian (*Durio Zibethinus*) and rambutan (*Nephelium lappaceum*). This national park consists of five main vegetation types such as alluvial forest, mixed dipterocarp forest, kerangas forest, submontane forest and secondary forest.

### 3.2 Sample Collection and Identification

10 standard mist nets were set up every day of sampling. The nets were set up around 5.00 p.m and will be checked for two hours interval. The last checked was at 11.30 p.m and the first check in the morning was at 6.30 a.m. The mist nets were set up across the stream, narrow pathway in the forest, trails, forest edge and the forest openings (Khan *et al.,* 2007). The identification of bats was based on Payne *et al.* (1985). The morphological measurements of bats were taken by using digital caliper and the weight was taken by using Pesola spring balance. The presence of embryo was detected by abdominal palpation (Funakoshi and Zubaid, 1997). Therefore, for every female captured, a check on pregnancy was done.

### 3.3 Specimen Preservation

Selected female individual were euthanized by using chloroform and dissected to examine the uterine horn. The bats and the embryos were preserved in 75% ethanol and deposited at the UNIMAS zoological museum.

### 3.4 Laboratory Work

The specimens of *C. brachyotis* from UNIMAS zoological museum were dissected to obtain the embryo of the bats or the developed uterine horns. There were 100 individual used in this project which includes some newly collected materials from field. Once dissected, the uterine horns of bats are observed and the presence of embryos or the developing in the size of horns was recorded. Three measurements of morphological features were taken such as Crown Rump Length (CRL), Greatest Length of Skull (GLS), and Forearm Length (FAL) were taken for three times to the nearest 0.01 mm and an average was derived. The measurements were taken for some representatives of embryos only. The picture of important parts of the embryo will be taken by using Nikon D3100 to improve picture quality for better identifying of prenatal process. The key anatomical features of the embryo were identified and compared. The features are very important for stages classification and ageing of the embryo. The embryos were staged by referring to Cretekos embryonic staging system (Cretekos *et al.,* 2005) and also referring to other publications (Wang *et al*., 2010; Nolte *et al*., 2009). The breeding and birthing time of *C. brachyotis* were estimated.

### 3.5 Data Analysis

Growth trajectories were established to see the relationship between three measurements and the body mass of the embryo. The growth trajectories were generated by performing two linear regression analyses on CRL, FAL and body mass against GLS respectively. All regressions were derived by using Excel Microsoft Word. The average total rainfall and temperature trends of Sarawak were used to relate with reproduction and birthing peak of *C. brachyotis*. Rainfall data were gathered from Sarawak Meteorological Department.

## 4.0 Results

### 4.1 Prenatal growth and development

A total of 100 female *C. brachyotis* were dissected and examined. All specimens were collected from various locations throughout Sarawak from year 1995 until year 2013. Some specimens were selected as representative to express the developmental series in this species. The specimens represented a clear developmental series in their development. Measurements of FAL of embryos ranged from 4.57 mm to 17.77 mm and were positively related to GLS ($R^2$=0.9697, Fig. 3a). Measurements of CRL of embryos ranged from 11.51 mm to 26.68 mm and were positively related to GLS ($R^2$=0.9215, Fig. 3b) less than was FAL.

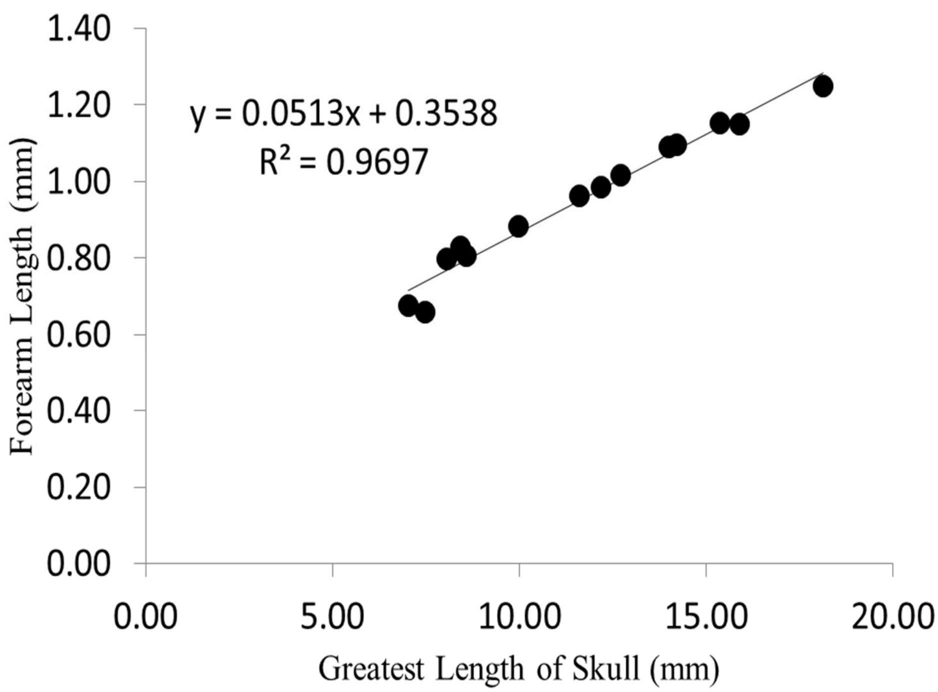


Fig 3(a). Regression analysis of growth in *Cynopterus brachyotis* for forearm length against greatest length of skull

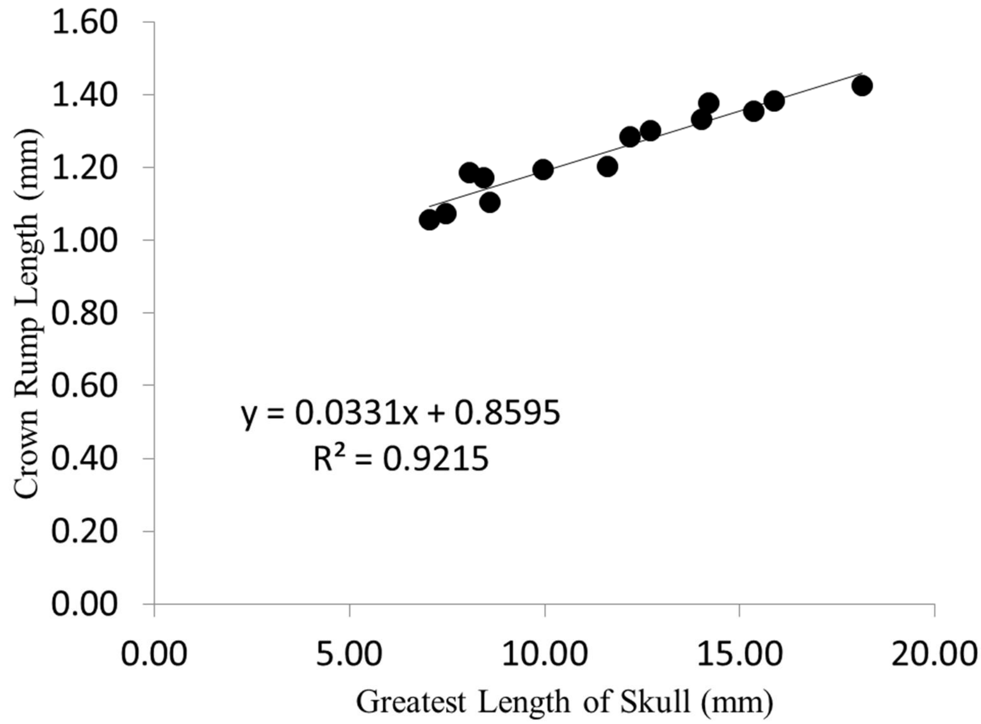


Fig 3(b). Regression analysis of growth in *Cynopterus brachyotis* for crown rump length against greatest length of skull

**4.2 Embryonic development**

Most of the females dissected were matured where 75 individuals are pregnant and 25 individuals are not pregnant. A total of 42 adult females are pregnant with minute embryo and 33 are pregnant with large embryo. A total of 33 individuals of developing embryo experiencing different stages of development are found in this study and classified into seven stages beginning at stage 14 and ending with stage 22; we did not have specimen for stage 16, and 21. Two embryos were classified in stage 14 and 18, six embryos match in stage 15 and five embryos in stage 17. For stages 19, 20 and 22, we had 7, 9 and 2 embryos respectively. One representative embryo photo from each stage is also provided for identification aid. The classification of the embryo into specific stages done by morphological examination and there may be a slight difference occurred in same stage.

Stage 14- Two specimens fit in this stage (Table 1).The presence of pigmentation in the retina of the eye can be detected, the shape of trunk is interrupted by cervical flexure and the tail is long and adjacent to head. The forelimb is elongated to be longer than its wide while the hindlimb become more obvious (Fig 4).

Stage 15- Six of the specimens are classified into this stage (Table 1). This stage can be classified by obvious change involving limb shape (Fig 4). The forelimbs are modified into foot plate formed but the tail is still adjacent to head area in early stage. Pigmentation of the eyes are more obvious than previous stage.

Stage 17- Five specimens collected fit in this stage (Table 1). The forelimb in this stage show great changes and the chiropatagium start to develop in this stage. The hindlimb interdigit tissue receding and the cervical flexure is absent (Fig 5).

Stage 18- This stage show further development in limb where the first digit is now free from the rest of hand plate while the tissue between all hindlimb digits disappeared. The overall appearance of the embryo is rounder and smoother and the eyelids do not covering most of the eyes (Fig 5).

Stage 19- There are seven specimens in this stage and the specimens show various difference in morphological growth (Table 1). Eyelids cover more of the eyes of the embryo. First digit of forelimb and all hindlimb digits develop knob-like claw with no brown pigmentation at base of claws (Fig 6).

Stage 20- Nine samples were classified in this stage (Table 1). The eyes are closed in all specimens. Zeugopod and autopod grow longer than stylopod in more advanced specimen. While, in the beginning of stage, the zeugopod length is quite similar to stylopod. The claws are shorter and light brown pigmentation at claws base. Besides, flexure at ankle joints is not really obvious. In more advanced specimen, pinnae are larger and lightly pigmented. Claws are more pointed at the tips and pigmented. In some specimen, there is pigmentation at noseleaf area in all sample, the dorsal trunk has not undergone pigmentation (Fig 6).

Stage 22- Only two specimens matched in this stage (Table 1). Pinnae are large and pigmented. Pigmentation around noseleaf area and hair pores also presence. In one sample, one of the eyes has begun to open again. Nasal process protruded away from nasal pit in both specimens. The wrists are either juxtaposed against the chin and the hindlimbs are crossed to each other and one of them extends towards the chin. The embryo resemble like an adult except it lacks fur and the pinnae do not stand erect, pinnae do not curled at tip and no fur at eyes and dorsal trunk areas (Fig 22). In MZU/M/161, long fur presence around eyes area and noseleaf area and very short hair at dorsal trunk. Tip of pinnae curled outward and pigmented while the dorsal trunk is pigmented.

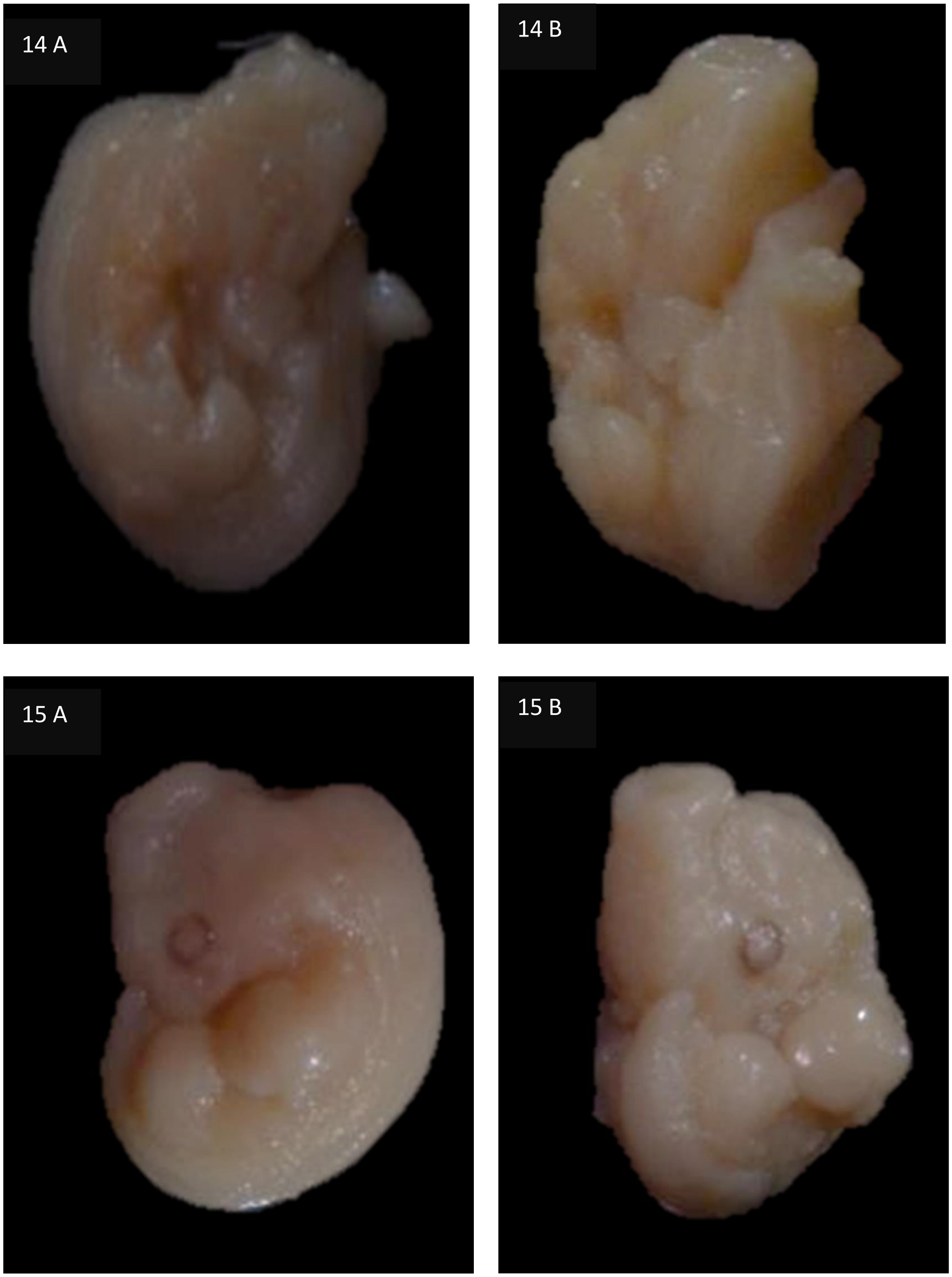


Fig 4. Stage 14 (MZU/M/1020) and stage 15 (MZU/M/1021). The first column shows lateral view and the second column shows ventral view. The number in the photo indicates the embryo stage

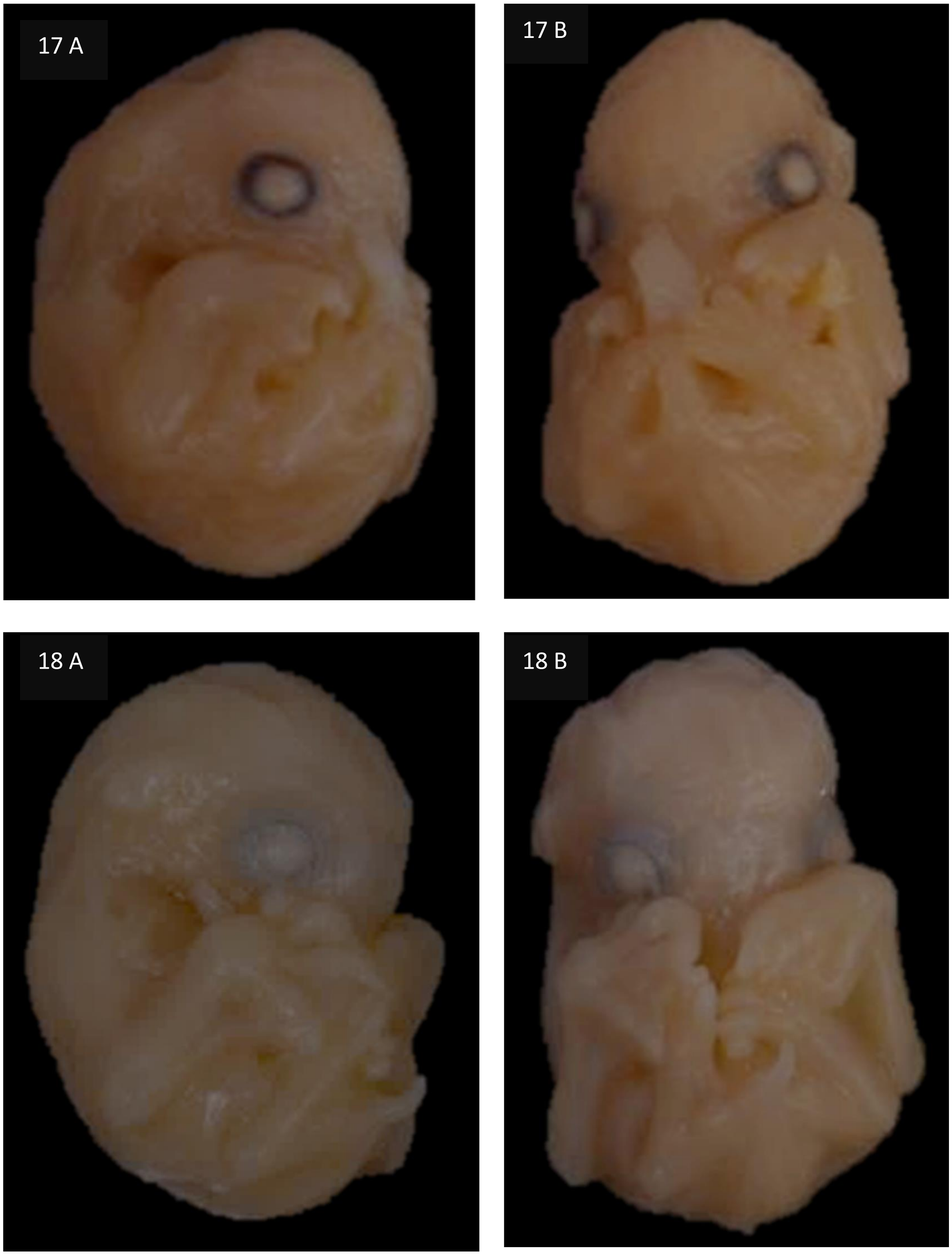


Fig 5. Stage 17 (MZU/M/1641) and stage 18 (MZU/M/2040). The first column shows lateral view and the second column shows ventral view. The number in the photo indicates the embryo stage

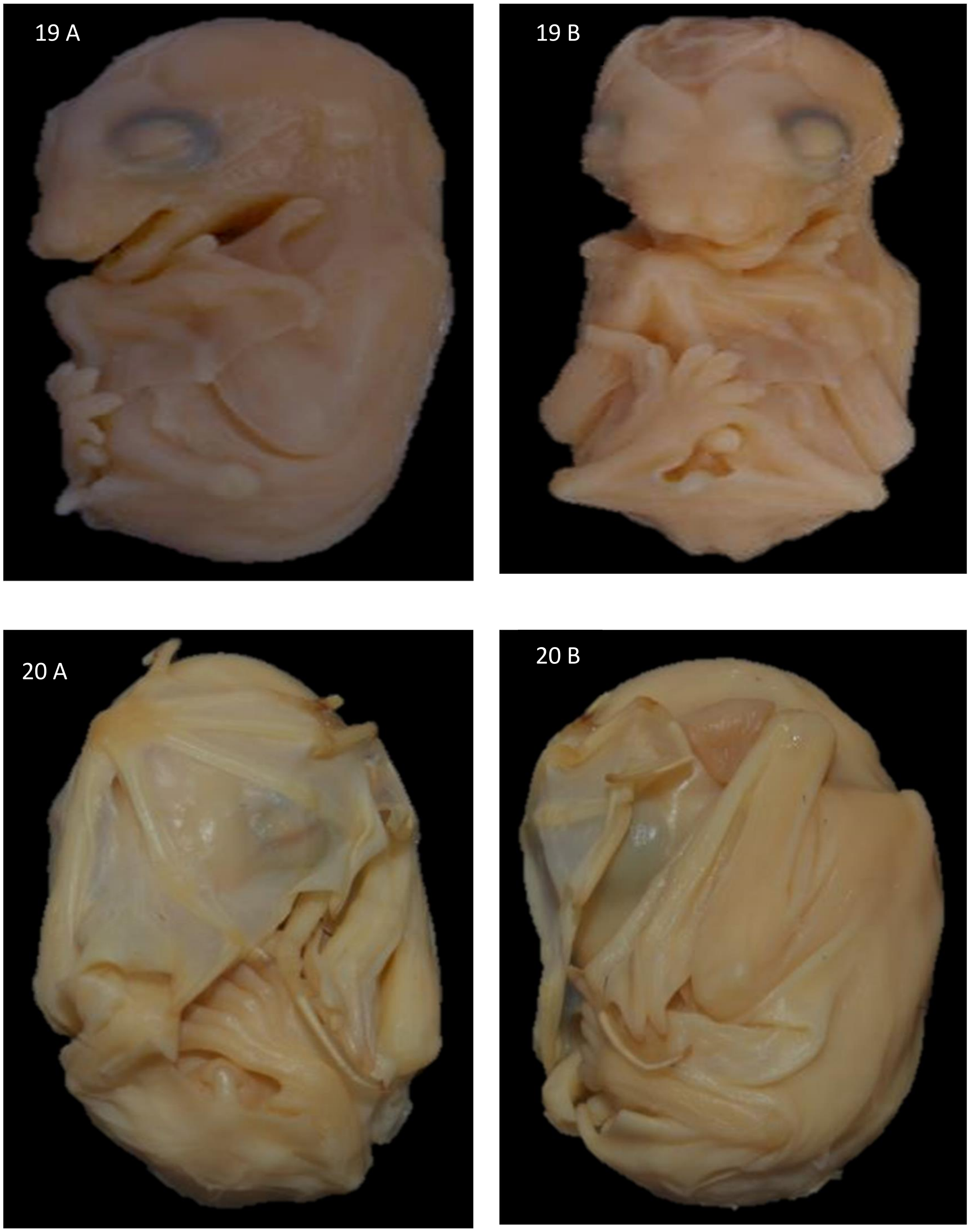


Fig 6. Stage 19 (MZU/M/2153) and stage 20 (MZU/M/988). The first column shows lateral view and the second column shows ventral view. The number in the photo indicates the embryo stage


Fig 7. Stage 22 (MZU/M/2199). The first column shows lateral view and the second column shows ventral view. The number in the column indicates the stage of the embryo

Table 1. The data of embryo profile for *Cynopterus brachyotis* throughout Sarawak

| Running no | Stage | dpc | Capture date | Locality | Expecting mating month | Expecting birthing month |
|---|---|---|---|---|---|---|
| MZU/M/159 | 18 | 60 | 1/6/2005 | Lawas | 4 | 7 |
| MZU/M/160 | 20 | 70 | 31/5/2005 | Lawas | 3 | 7 |
| MZU/M/161 | 22 | 80 | 1/6/2005 | Lawas | 3 | 7 |
| MZU/M/340 | 19 | 65 | 2/1/2009 | UNIMAS | 10 | 2 |
| MZU/M/357 | 19 | 65 | 30/8/2005 | Bako NP | 6 | 10 |
| MZU/M/988 | 20 | 70 | 9/9/2006 | P.Satang Besar | 6 | 10 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MZU/M/989 | 15 | 46 | 12/9/2006 | P.Satang Besar | 7 | 11 |
| MZU/M/993 | 20 | 70 | 8/2/2006 | Mt. Penrissen | 12 | 3 |
| MZU/M/1007 | 15 | 46 | 27/6/2005 | Batang Ai | 5 | 9 |
| MZU/M/1009 | 20 | 70 | 28/6/2005 | Batang Ai | 4 | 8 |
| MZU/M/1020 | 14 | 44 | 11/12/2006 | Batang Ai | 10 | 2 |
| MZU/M/1021 | 15 | 46 | 14/12/2006 | Batang Ai | 10 | 2 |
| MZU/M/1030 | 20 | 70 | 12/12/2006 | Batang Ai | 10 | 2 |
| MZU/M/1408 | 20 | 75 | 28/10/2012 | Sebangkoi RP | 8 | 12 |
| MZU/M/1418 | 19 | 65 | 30/5/2005 | Bako NP | 3 | 7 |
| MZU/M/1634 | 15 | 46 | 7/12/2004 | Niah NP | 10 | 2 |
| MZU/M/1641 | 17 | 54 | 31/7/1996 | UNIMAS | 6 | 10 |
| MZU/M/1666 | 17 | 54 | 29/7/2003 | Bau | 6 | 10 |
| MZU/M/1668 | 19 | 65 | 17/4/1995 | Barrio | 2 | 6 |
| MZU/M/1671 | 17 | 54 | 24/6/1996 | Penrissen | 5 | 8 |
| MZU/M/2040 | 18 | 60 | 9/6/2008 | Taan Plantation Sibu | 4 | 8 |
| MZU/M/2049 | 15 | 46 | 9/6/2008 | Taan Plantation Sibu | 4 | 8 |
| MZU/M/2117 | 20 | 60 | 31/10/2012 | Niah | 9 | 12 |
| MZU/M/2148 | 15 | 46 | 14/2/2007 | Samarakan Bintulu | 12 | 4 |
| MZU/M/2149 | 17 | 54 | 20/12/2007 | Samarakan Bintulu | 10 | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MZU/M/2153 | 19 | 65 | 14/12/2007 | Samarakan Bintulu | 10 | 2 |
| MZU/M/2158 | 14 | 44 | 15/12/2007 | Samarakan Bintulu | 11 | 3 |
| MZU/M/2169 | 19 | 65 | 16/3/2012 | Batang Ai | 1 | 4 |
| MZU/M/2170 | 20 | 70 | 1/2/2012 | Mulu NP | 11 | 3 |
| MZU/M/2177 | 17 | 54 | 27/10/2008 | Murud | 9 | 1 |
| MZU/M/2199 | 22 | 80 | 19/8/2008 | Bau | 6 | 9 |
| MZU/M/2226 | 19 | 65 | 20/8/2008 | Bau | 6 | 9 |
| MZU/M/2744 | 20 | 80 | 27/5/1996 | Samunsam WS | 3 | 7 |

### 4.3 Breeding status

In this study, I estimate the reproduction of *Cynopterus brachyotis* for several years from various localities in Sarawak. *Cynopterus brachyotis* is characterized by two peaks birthing season which are in February and from July until October. At the same time, a small subset of birth may occur in every month except for May (Table 1). The percentage of birth time in this species is made by referring on estimation of day post coitum in every embryonic stage (Cretekos *et al*., 2005). The generalization made here is based on estimation of four month gestation period of this species (Heideman, 1998; Nowak, 1999). The breeding of this species occur in all year round with two peaks which are in June and October (Fig 8). The swollen uteri indicating pregnancy is not consistent where most of them occur in April and June, while the percentage in May is much lower. It is obvious that the breeding pattern of this species does not restrict to seasonality. Thus, it is clear that *C. brachyotis* reproduction can be categorized as aseasonally polyoestrus. The peak incidence of this species does not correlate with greater rainfall season which is from November until January. Instead, the birth peaks were timed with lesser rainfall season and low temperature especially in February. Meanwhile, the second peak of birthing period was in higher temperature. The peak breeding time was recorded in June and October which are also during lesser rainfall season and high temperature. The inconsistent swollen uteri of *C. brachyotis* in Sarawak is further strengthen the earlier assumption made where this species appears to be aseasonally polyoestrus. It gives a picture that this species breed at any time and do not depend much on their environment.

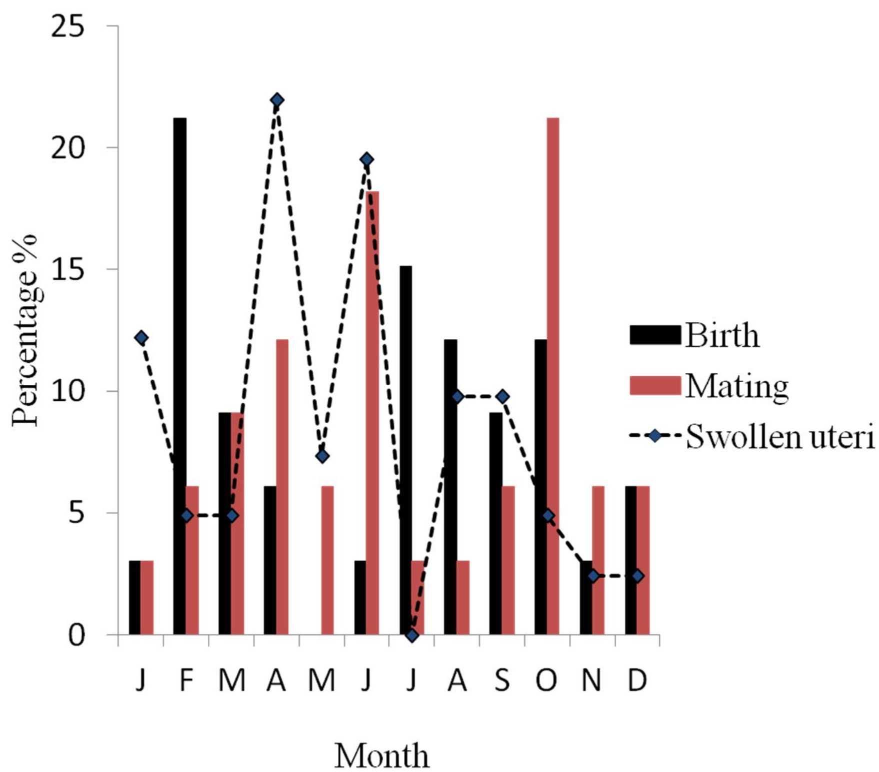


Fig 8. Percentage of reproduction in *Cynopterus brachyotis* throughout the year

## 5.0 Discussion

This study provides the first description on prenatal growth and development of *C. brachyotis*. The previous study by Wyant and Adams (2007) in molossid bat *Mops condylurus* showed that forearm length (FAL) is positively related to greatest length of skull (GLS). The regression of crown rump length (CRL) against GLS was also positively related but less closely than FAL.The regression in *C. brachyotis* also gives similar result as in *M. condylurus*, except that there is a bit difference in $R^2$ value of each regression. In *M. condylurus*, the $R^2$ value for FAL against GLS is 0.988 while for *C. brachyotis* is 0.9697. For the regression of CRL against GLS, the $R^2$ value for *C. brachyotis* is 0.9215 which is lower than 0.979 as recorded in *M. condylurus*. The difference in both species is perhaps due to both species are from difference family.

This study tried to illustrate and described the embryo development characteristic of *C. brachyotis* for the first time. The size of pregnant bat uterine horns is the best initial indication in determining pregnancy and stage of gestation (Badwaik and Rasweiler, 2001; Wang *et al*., 2010). But, for voucher specimens that have been stored for many years, the diameter and length of the uterine horn may altered. Some specimens in same stage may show difference development can be categorized as early or late stage (Cretekos *et al*., 2005).

Although some publications on reproduction are available for this species (Lim, 1970; Kofron 1997), this project is the first account, to my knowledge, examines the pregnant status by staging the embryo based on current staging system (Cretekos *et al*., 2005) and other publication on embryonic description (Nolte *et al*., 2009; Wang *et al*., 2010). In the presence study, *C. brachyotis* reproductive pattern is characterized by aseasonally polyoestrus. Bumrungsri *et al*. (2006) characterized reproduction of this species in Thailand as seasonal bimodal polyoestry with postpartum oestrus, while Kofron (1997) reported that *C. brachyotis* reproductive pattern is characterized by seasonal and continuous bimodal polyoestry. At the same time, Ingle (1992) mentioned that there are two birth periods per year in this species on the Philippine island of Luzon. Such variation in latitude difference is one of the contributors to the difference in *C. brachyotis* breeding pattern as it determines the seasonality of rainfall and food availability (Bronson, 1989; Racey and Entwistle, 2000). On the other hand, Lim (1970) found that the pregnancy peak of *C. brachyotis* was in January, May and September. Meanwhile, aseasonally polyoestrus reproductive pattern in *C. brachyotis* was shown in Ulu Gombak where the pregnant females are caught at almost every month (Funakoshi and Zubaid, 1997) which is at the same latitude as (Lim, 1970). Furthermore, fetuses of this species are reported in every month (Lim, 1970). These reports show that the breeding pattern does not associate solely on latitude, and the reproductive strategy may differ at different locality. In this study, the birth peaks were in February until March and from July until October with small birth subset occur in other months. This result is quite similar as a report by Kofron (1997) involving *C. brachyotis* and *Cynopterus minutus* whereby birth period was from mid-January to mid-April and from mid-June to early October. Both studies showed weak correlation between birth periods and greater rainfall season. The temperature also indicates weak association with birthing peaks of this species as the mean temperature in February is much lower than in July until October.

The peak birthing seasons of *C. brachyotis* appears not to be linked with flowering/fruiting and availability of specific plant species too. It is also known that in Malaysia, the fruiting season is between March and June (Lim, 1970).This shows that this species is not depending on specific food and able to feed on various types of fruit (Mohd-Azlan *et al.*, 2010; Tan *et al.*, 2000). Besides, it is known that this species likely to consume *Ficus* spp. which are available throughout the years (Tan *et al*., 2000).

## Conclusion

Finally, this study is important as it provides first description on prenatal growth, embryo staging series and breeding pattern of *C. brachyotis* through embryo staging examination of specimens throughout Sarawak. In prenatal growth of *C. brachyotis,* it is showed that the growth of FAL and CRL are positively related to the growth of GLS. Although the development stage that I managed to present here was not complete, it is such important information to us that the current staging system can fit into this species even the bat used as a model of staging system is from different families. This study was giving wide information regarding reproductive ecology of this species. Robust data that I have here is enough to tell the picture of breeding pattern of *C. brachyotis.* The breeding pattern of *C. brachyotis* is aseasonally polyoestrus as the female may breed more than one per year at any time without depending on environmental condition. Rainfall and temperature are not affecting the reproductive activity of this species as mating and birthing may occur in any month of the year. In the future, we may have more data from each division in Sarawak to give better information and the staging of the embryo should be done by using more advance microscope to examine early stage of development.

The growth trajectories done here may not able to describe prenatal growth wholly. In future, we may add more parameter such as wing area, body mass and quantifying pattern of skeletogenesis. Besides, more individual embryo can be used in constructing growth trajectory to give more accurate result. Other than that, the staging of the embryo should use advance microscope and better camera for taking embryo photo. It will be a better result if we have more specimen for each division in Sarawak as it may tell us about how geographical difference and habitat preference correlation with the reproductive activity.

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